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PHOTOENERGETICS OF A SYNCHRONOUS CHLORELLA CULTURE

[Following is a translation of an article by L.N. Bell, Ye.A. Lin'kova, G.A. Slobodskaya, K.S. Spektorov, Ye.P. Fedenko, and G.S. Bukina (Institute of Plant Physiology imeni K.A. Timiryazev, Academy of Sciences USSR, Moscow) from the Russian-language periodical *Fiziologiya Rasteniy* (Plant Physiology), Vol. 14, No.5, Moscow, September-October 1967, pages 866-871.]

1. INTRODUCTION

The maximum energy production in photosynthesis determines its potentialities as an energy storing process. The concrete value of the energy production that is measured depends on a whole series of external and internal factors. The combination of external conditions (light, temperature, CO_2 concentration) that are best for energy production or efficiency has been explained to a considerable extent. However, the connection between a plant's energy efficiency and its internal states and especially the particular structural characteristics of its photosynthetic apparatus are far less known.

Undoubtedly, the structure of the photosynthetic apparatus varies according to the degree of cell development in the photosynthesizing organs. The efficiency of its operation consequently changes accordingly. Plant photosynthesizing efficiency may thus depend on the age of the photosynthesizing cells. An investigation of this relationship is the purpose of this work.

It has become possible to perform such research due to the development of the technique of growing synchronous cultures of unicellular photosynthesizing algae.

There are several works covering the study of the intensity of photosynthesis in relation to the developmental phase of synchronous cultures of unicellular algae (1-5). However, the data of these works are conflicting and it is difficult to compare them with one another. This may be explained by the fact that the numerical expression of "photosynthetic activity" depends upon the selection of values (dry weight, chlorophyll content, cell number, etc.) that represent this activity. But these values themselves change as the culture grows. Such indeterminacy may be avoided if the efficiency of photosynthesis is expressed by the quantum or energy yield. These values characterize the efficiency of utilization of each quantum absorbed and are consequently of the highest interest from the viewpoint of studying the mechanism that regulates the storage of light energy during photosynthesis.

In view of the fact that a certain discrepancy may as a rule occur between gas exchange and energy exchange, plant energetics should be studied by methods which investigate energy directly (6).

A study has been made in this work of the dependence of photosynthetic energy yield on the phase of cell development. The energy efficiency ε denotes the ratio of the amount of energy stored during photosynthesis for a certain period of time to the amount of energy absorbed by the plant during this time. Ordinarily, one of the main difficulties in the experimental determination of ε is the complexity of measuring the absorbed energy flow. This circumstance makes it hard to take a large number of measurements, which are absolutely essential in experiments of this type, and hence due to the inconstancy of the properties of the subject being studied and its sensitivity to many factors there is a large scattering of experimental data.

For this reason, it would be very convenient to present a method for conducting such tests, which has been worked out by one of the authors of this article. The method allows a direct determination of the energy efficiency without special measurement of the flow of absorbed radiation (7).

2. METHOD

The method referred to (temperature curve) will consist of the following. The temperature of an algae suspension is measured under different light intensities. It may be shown that at light intensities that correspond to a linear segment of the photosynthesis light curve, the energy efficiency ε is determined

$$\varepsilon = 1 - \frac{\operatorname{tg} \varphi_0}{\operatorname{tg} \varphi_\infty}, \qquad (1)$$

where φ_0 is the angle of inclination of the light curve at low light intensities (corresponding to the linear part of the photosynthesis light curve) and φ_∞ is the angle of inclination of the temperature curve at high light intensities that correspond to photosynthesis saturation (for details of the method see work (7)).

The experimental subject was a thermophilic strain of unicellular green algae Chlorella pyr.Pringsh. 82T. The initial synchronous culture was gotten by natural sedimentation (8).

The autospores obtained by this method were transplanted from time to time to a fresh cultural medium, on which the cells spent 2-3 subsequent cycles of synchronous development under conditions optimum for the given strain: light intensity was 10 000 lux, the culture chamber temperature was 37-39°, air mixed with 0.08-0.1% CO_2 was blown in, and the light to darkness ratio was 7:17 hours. The initial autospore density was approx. $5 \cdot 10^6$ cm^{-3}.

It was shown in (9, 10) that the first synchronous development cycle of a culture on fresh medium is not characteristic for the given strain and always varies somewhat from all subsequent cycles of synchronous development. Hence, the cells require some time to adapt to the fresh medium. Consequently, after the autospores were transplanted to a fresh culture, the cells passed through one cycle of synchronous development in this medium before the beginning of the experiment. The experiments were therefore carried out in the second and subsequent cycles of synchronous development.

The culture was not darkened for 7 hours of development during the experiments to measure ε, although it was continuously illuminated up to the production of new autospores. Under these circumstances the production of autospores from maternal cells set in in about 10 hours after the beginning of illumination of the initial autospores. The multiplication factor, i.e. the average number of autospores forming in the medium from a single maternal cell was approx. 20.

To determine the photosynthetic energy efficiency out of the culture chamber, samples ranging in size from 3-15 ml of suspension were taken in relation to their optical density which increased in proportion to the growth of the culture.

The suspension selected to measure ε was centrifuged at a rate of 2000 revolutions per minute for 10 minutes. The cells were then resuspended in a Warburg 9 buffer solution, whereupon the cell density following centrifuging was chosen so that the absorption factor for the suspension was identical for all tests. This was achieved by a specially made photoelectric densitometer composed of a silicon photoelectric cell with a large surface. The photocell registered a large part of the light that was not absorbed by the suspension, placed in a glass cup having the same form and size as a photocalorimeter. The absorption factor of the suspension was approx. 50%.

Approximately 40 minutes passed from the time the suspension was removed from the culture chamber to the beginning of the ε determination experiments (the measurements themselves took approx. 30 minutes). Consequently, some phase displacement took place in cell development at selection and upon measurement. As centrifugation and adjustment of the cell absorption factor occurred in darkness or in weak light, this shift was small in comparison with what occurred in the culture chamber which was subjected to strong emission. This shift was evidently larger in the later stages of the developmental cycle, where the cells can also develop without light. We did not determine the amount of shift, and therefore always further on give the time which passed from the beginning of illuminating the autospores to the time the cells were moved from the chamber.

After each selection a part of the cells were examined under the microscope. Morphological changes in the cells resembled those which were earlier observed from the same strain (11). There occurred merely some prolongation of the general developmental period of the culture for about one hour, probably caused by the application in our experiments of somewhat lower light intensity (10,000 lux instead of 18,000) and lower temperature (38° instead of 40°).

In the first series of experiments the energy efficiency was determined by light intensities lying within the range from 200 to 1700 erg/cm^2/sec. In four tests measurements were taken for a whole cycle of development, i.e. up to the moment of producing all autospores from the parent cells, which occurred in 14 hours after the beginning of illumination of the autospores. A large number of short tests were run on a 0, 2, 4, 6 hour schedule after the beginning of illumination of the autospores, i.e. for a period of 6 hours. This series of tests was run to determine the specific course of ε during the first stages of development. In all these tests the light used for measurements in the photocalorimeter were produced by an incandescent lamp and a system of light filters made of a water solution of $CuSO_4$, as well as S3S14 and ZhZS6 glass filters.

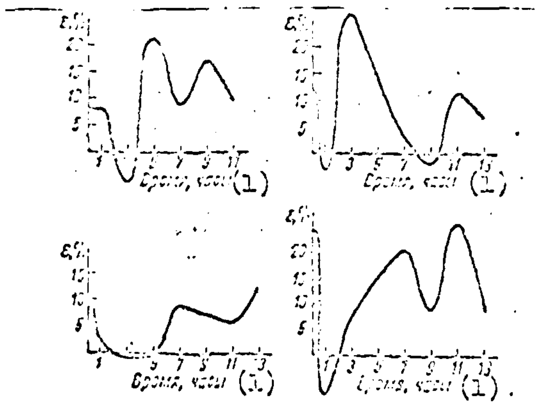
In a second series of tests measurements were made in light intensities ranging from 1700 to 3000 erg/cm^2/sec, i.e. at light intensities that exceed compensation. In these tests incandescent lamp light was passed through a water solution of $CuSO_4$ and S3S14 and BS8 glass filters. The spectral composition of the light consequently differed somewhat from the light composition used in the first experiments.

EXPERIMENTAL RESULTS

The results of the four tests covering a whole cycle of development are shown in the Figure.

As shown by the Figure, the curves displaying the relation of ε to the stage of development had the following general characteristics: 1) a sharp drop in ε after 1-3 hours of illumination in comparison with the value of ε for autospores; 2) an increase in ε for subsequent cell development; 3) the occurrence of a number of less sharply pronounced maxima and minima.

The low minimum observed during the first hours of illumination is most interesting. This minimum was also investigated in the short test series (maximum illumination time was 6 hours). A drop was seen in 11 cases out of 13.



Dependence of the energy efficiency of photosynthesis on cell development stage in a synchronous Chlorella culture

Abscissa - autospore illumination time; ordinate - energy efficiency

1 - time, hours

We tentatively think that a drop takes place in cases were the absolute value of ε was less than nearby values by at the most 5%, which with a 15% average value of ε in the autospores means a 33% yield in the relative value of ε. It is possible that the negative values of ε that were observed are real. The negative values of ε mean that in the selected area of light intensity photo-induced exoergonic processes occur with greater intensity than photo-induced endergonic processes (or roughly: photorespiration is more intensive than photosynthesis).

Various experimental controls were set up to explain the occurrence of the observed drop.

Experiments of two types were made. In the first series of controls run on the same 2-hour schedule a determination was made of the "energy efficiency" of an inactive liquid -- ink suspension or dye solution. These tests actually characterized the precision of photocalorimeter readings under light intensities used in the experiments.

It was shown that on the average (running four measurements per one test) ε differed from zero by a total of

2-3%, although in individual measurements rather large discrepancies were sometimes obtained and in nine cases out of the total 22 tests, the "effect" was observed, i.e. the difference in efficiencies was over 5%. In 12 tests this effect did not occur and in one case the opposite effect was observed.

Therefore, at the same time as in the algae culture tests a drop was observed in 11/13=85% of the cases, in tests with the liquid it was observed in a total of 9/22=40% of the cases. Moreover, the absolute amount of the drop, i.e. $\varepsilon_1 - \varepsilon_2$ and $\varepsilon_5 - \varepsilon_2$ (the subscript indicates the number of the test; the first measure refers to the autospores), was noted to be higher in experiments with algae and ordinarily 10-15%, whereas in the tests using inactive liquids it was in the order of 5%.

We are consequently inclined to think that the energy effectiveness of photosynthesis in the cells of a synchronous Chlorella culture at nearly compensating light intensities, obtained as a result of irradiating autospores for 1-3 hours, is markedly less effective than for autospores or cells at a later stage in development.

The certainty of a drop in ε measured during the first hours of illuminating the cells does not by any means imply a certainty in the drop of initial energy efficiency of photosynthesis. This may be explained by the fact that, as seen from formula (1), a low value of ε might be brought about by a decrease in $tg\varphi_{0}$, i.e. a slope in the heating curve at light saturation intensities. This might have taken place when the suspension faded at the given light intensities, and the slope is lower the smaller the absorption coefficient. A second series of controlled experiments were run to check out this possibility. Using an SF-10 spectrophotometer, the transmittance of the suspension was measured after the cells were irradiated with saturation intensities for the same time as in the original experiments. Moreover, the kinetics of temperature change in the suspension illuminated with constant light intensity was studied in the same calorimeter (the temperature should be reduced in case of fading). Finally, a study was made of the kinetics of change of transmittance of the suspension in a transparent vessel that had the same size as the calorimeter vessel. Changes in the absorption factor were judged on the basis of readings of the large area photocell lying in back of the vessel. The experiment showed that the use of light saturation intensities does not produce substantial changes in the absorption coefficient.

It may be thought that changes in the absorption coefficient, if they take place, arise practically spontaneously after variations in light intensity and therefore are not caught by the methods which were utilized. This possibility has been disproven by measurements made on a differential spectrophotometer (12). The rapid reversible changes observed in the absorption coefficient never exceed 1%. Consequently, variation in ε should not be regarded as the result of cell fading when irradiated in the calorimeter.

As has been said, measurements of ε in the tests under consideration were made at low light intensities (200-1700 erg/cm^2/sec), which are close to compensating intensities (according to measurements made in an amperometric device (13) with a vessel similar to the one used in the photocalorimeter, compensation taking place at 1200 erg/cm^2/sec.). A second series of tests was then run using a suspension of algae at higher light intensities, succeeded compensation intensities, in the range of from 1700-3000 erg/cm^2/sec. Out of the seven tests using suspensions with an average value of ε = 15%, only in two did one observe a slight drop in the order of 5%, and merely in two out of 19 control tests with an inactive liquid was a drop observed. These tests consequently showed that at the intensities of light measured which exceed the compensation values, the energy efficiency does not significantly depend on the stage of development of the cells, and in any case the cells were exposed for the first six hours. Particularly, a reduction (drop) in ε was not observed as a result of irradiating the autospores.

CONSIDERATION OF RESULTS

A reduction in energy efficiency ε in young Chlorella cells in comparison with ε in autospores was observed by us only at low light intensities which lie in the same order as those of photosynthetic compensation by respiration.

In this area of intensities the effect of light on respiration may be especially noticeable, which is confirmed by the existence of the Kok effect (14, 15) and experiments on oxygen absorption in light (6, 17). It is therefore only natural to assume that the drop in ε in the field of low light intensities is also caused by the change in respiration in light.

Of particular interest in this connection are the results of Sorokin and Myers (18), from which one may conclude that the respiration increase in light may be greater in young cells of thermophilic Chlorella strains than in the older ones.

At rather high light intensities the dependence of respiration on light intensity may possibly be less drastic, and this might explain the independence of $\mathcal{E}$ on the stage in this field of light intensity.

In the general case it should be expected that the form of photosynthesis light curve may vary with a change in the developmental stage. Such a deformation of the light curve may be produced by a non-uniform variation in the effectiveness of photochemical and enzymatic reactions of photosynthesis. In particular, as special measurements which we made on an infrared gas analyzer have shown, the sharpest changes in the intensity of photosynthesis at light saturation intensities were discovered at a late stage in development, specifically during the period of autospore formation. At this stage (approx. 10-11 hours) the intensity of CO_2 absorption was at a minimum, at the same time as $\mathcal{E}$ was at its maximum.

On the other hand, it should be emphasized that the data which we obtained on the course of energy efficiency did not, generally speaking, necessarily have to coincide with the corresponding data for quantum yield, as the degree of connectedness between the gas exchange and energy metabolism processes may also depend upon the developmental phase. The data obtained on energy efficiency have a direct relation to the problem, determining the maximum energy production of photosynthesis. They show that at low light intensities the value $\mathcal{E}$ measured may depend upon the stage of cell development and, consequently, in the case of working with a non-synchronous culture may also depend upon the distribution of cells according to phase (4). According to our data, at rather low light intensities, the measured value of $\mathcal{E}$ was larger in autospores than in cells during the following stage of development, i.e. those that were illuminated 1-3 hours. It is possible that this may partially explain the wellknown method of preparing plants for the measurement of quantum yield by holding them in the dark for a prolonged period before testing. A considerable proportion of the cells of algae prepared in this manner would become synchronized and a larger part of the cells may be in the autospore phase. If the measurement of ε would be taken at somewhat higher light intensities, such a preparation may be superfluous, because ε under these circumstances does not depend upon the stage of development according to the results which we obtained.

We note in conclusion that the regularities which we observed in the change of ε at varying cell developmental phases can hardly be regarded as universal. A variation in the energy exchange of the cells at different developmental stages may be determined by peculiarities of the plant species, the conditions in which they are cultivated, i.e. their prehistory and possibly even by the handling of the plants while preparing them for measurement (11, 19).

CONCLUSIONS

1. The energy efficiency of photosynthesis (ε) was measured in a photocalorimeter as a function of cell developmental phase in a synchronous Chlorella culture.

2. A number of maxima and minima of ε were discovered at light intensities below the compensating point. The minimum of ε was especially clearly pronounced in 1-3 hour old cells. At light intensities somewhat higher than the compensating point, this drop was not observed.

3. It is assumed by comparing the data obtained with the literature that the drop in ε observed in young cells at subcompensating intensities is caused by influence of light on energy degradation processes (photo-induced exothermic reactions).

4. The relatively high value of ε in autospores as compared with the value of ε in cells illuminated for 1-3 hours may explain the widespread process of preparing algae for quantum yield measurements.

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